

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Male Reproduction: One of the Primary Targets of Bisphenol A

Tomáš Jambor, Bistáková Jana, Greifová Hana,
Tvrdá Eva and Lukáč Norbert

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.68629>

Abstract

Infertility is a major health issue affecting human life. The most notable factors causing male infertility is exposure to environmental contaminants. Bisphenol A (BPA) is a common toxic environmental contaminant. Human population is exposed to bisphenol A through air, water, food and a variety of industrial products. Growing evidence from research on laboratory animals supports the hypothesis that bisphenol A is able to adversely affect male reproductive function. The specific mechanisms of action of bisphenol A are wide but not definite. Bisphenol A interferes with the hormonal metabolism and regulation, binding affinity or enzymatic activity, resulting in a deviation from a normal reproductive behaviour. Binding ability to androgen and oestrogen receptors, as well as other properties, is currently investigated. A decreased sperm count, inhibition of sperm motility and reduction of organ weights were observed and linked with oxidative stress after bisphenol A treatment. In addition, prenatal exposure to bisphenol A may lead to adverse effects in the offspring. In this review, we address the topic of BPA effects on male reproductive function and emphasize its effects on testicular steroidogenesis and spermatogenesis. A considerably more detailed and systematic research focusing on bisphenol A toxicology is required for a better understanding of risks associated with exposure to this endocrine disruptor.

Keywords: reproduction, male, bisphenol A, steroidogenesis, spermatogenesis

1. Introduction

Over the last decade, research has focused on the potentially hazardous effects of a wide range of chemicals present in the human or wildlife environment. An increased occurrence of male reproductive and developmental disorders such as hypospadias, cryptorchidism and

testicular cancer as well as a decreased semen quality have been related to the action of endocrine disruptors. Endocrine-disrupting effects of commercially available products have the potential to cause reproductive dysfunction alongside with adverse effects on development and sexual differentiation. The group of known endocrine disruptors is extremely heterogeneous. One of the most common environmental contaminants classified as endocrine disruptors is bisphenol A (BPA). Many studies have defined BPA as hazardous to the health of humans and animals, particularly to male reproduction [1]. BPA plays a key role in testicular disorders, due to its oestrogenic properties. Oestrogen biosynthesis takes place in the testicular cells; hence the absence of oestrogens causes negative effects on male reproduction [2]. Physiological levels of oestrogens are essential for a normal spermatogenesis; however, overage of oestrogens together with a deficiency of testosterone may cause infertility [3]. In addition, some reports have shown that BPA behaves as an androgen receptor antagonist, interrupting normal androgen receptor binding activity and interactions between androgen receptors and endogenous androgens. Such effects by BPA on the function of endogenous androgens could interfere with normal processes of spermatogenesis, which are controlled by numerous endogenous hormones [4, 5]. Moreover, androgens play characteristic roles in the expression of the male phenotype, development and maintenance of the secondary male characteristics and regulate the expression of an array of target genes that are important for a proper male fertility [6]. As such the chemical substances with antiandrogenic properties can react with male sexual functions and behaviour by blocking the binding of androgens to androgen receptors and a following induced expression of gene by androgen. It has been reported that BPA has adverse effects on the male reproductive system including a decreased sperm count, abnormal sperm motility and reduced reproductive organ weights [7]. One of the potential mechanisms of action of BPA on male reproductive functions and sperm quality has been also proposed to act through oxidative stress. Environmental contaminants such as BPA have been shown to induce reactive oxygen species overgeneration in both intracellular and extracellular spaces leading to cell death and tissue injury [8]. Sensitivity to BPA is not the same at all stages of life, and there are specific critical phases of male development that are more vulnerable to BPA exposure [9]. One such sensitive phase wherein organ differentiation and development take place is the prenatal and perinatal period. The cumulation of BPA in tissues of the male reproductive system is associated with different pathological consequences since low-level BPA exposure during embryonic phase of life has been observed in reduction of effectiveness of spermatogenesis in male descendants [44]. Many experiments have examined the effect of prenatal, neonatal and lactational exposure to low BPA doses. Such studies examined the impact of small dosage of this endocrine disruptor throughout crucial stages of development in various cells and organs. These crucial stages continue throughout reaching of sexual maturity, the physiological phase of modification to fertility [10]. In relation to male reproductive functions, sexual dysfunction in animal studies is difficult to conduct. However, changes in sexual behaviour including a reduced performance in latency and frequency of intromission among rodents exposed to BPA have also been reported [10, 11]. Even results from a human study involving workers of BPA manufactures in China from 2004 to 2008 provide important evidence that occupational exposure to BPA significantly increases the risk of male sexual dysfunction [12]. Another issue that is becoming increasingly debated in the

context of male reproductive function and endocrine-disrupting compounds, such as BPA is their ability to modify the epigenome. Hormone cascade pathway is usually returnable and activates constant modulations of cell processes. Throughout sexual development, sex steroids are able to initiate persistent impact on activities of gene that induce developmental changes of cells and genes to react to another hormonal impulse during life. This hormonal imprinting or gene programming probably include mechanisms of epigenetics related to DNA methylation, which can be transmitted from mother cell to daughter cells and cause permanent changes [13]. Multiple evidence from *in vitro* and *in vivo* models have established that epigenetic modifications caused by in utero exposure of BPA can induce alterations in gene expression [14]. Currently, over 2.7 million metric tons of BPA are produced annually, primarily to be used in the production of polycarbonate plastics. This product is a constituent of a wide variety of products, including plastic packaging, water or milk bottles, food wrapping and food cans. BPA can leach into food or beverages from plastic containers and has been found in various human food samples. We may conclude that humans are exposed continuously to BPA primarily through diet [15, 16]. We considered these facts as crucial in the context of a mutual relationship between BPA and potential modifications in male reproductive system.

2. Potential impact of BPA on the steroidogenesis

There is overwhelming evidence about the potential ability of BPA to affect cellular processes, such as steroidogenesis and spermatogenesis. The testicular compartments responsible for steroidogenesis and spermatogenesis are the seminiferous tubules and interstitium. Both are morphologically distinct but functionally connected. Steroidogenesis and spermatogenesis are two vital, high energy demanding processes which are exceptionally vulnerable to damage caused by BPA [17]. Steroidogenesis is a process underway in the Leydig cells. Testosterone as a product of the steroidogenic pathway is released from the Leydig cells under the control of the luteinizing hormone (LH). LH binds to the LH receptor to induce the dissociation of the α subunit of the G protein. G_{α} then activates the cyclic adenosine monophosphate (cAMP). cAMP binds to protein kinase A (PKA). The active PKA phosphorylates certain cytoplasmic proteins, which, in turn, will increase the transportation rate of cholesterol into the inner mitochondrial membrane. Cholesterol is then catalyzed by the $P450_{SCC}$ enzyme into pregnenolone. Pregnenolone is delivered to the smooth endoplasmic reticulum and subsequently converted into testosterone. Depending on the species, this conversion can occur via progesterone, 17α -OH progesterone and androstenedione through delta-4 intermediates or via 17α -hydroxypregnenolone, dehydroepiandrosterone and androstenediol as delta-5 intermediates by the actions of enzymes, 3β -hydroxysteroid dehydrogenase (3β -HSD), 17α -hydroxylase and 17β -hydroxysteroid dehydrogenase (17β -HSD) [18]. BPA can alter the level of endogenous steroids at a particular site by altering its synthesis, metabolism, distribution or clearance. Alternatively, the chemicals may interact directly with the steroid receptor to either mimic or block steroid actions [19, 33]. Hormonal activity is mediated by binding to steroid receptors. Specific hormone-receptor complex is translocated to the DNA molecule.

After this step alterations in the expression of steroid—responsive genes—are observed [20]. BPA is able to inhibit the steroidogenic process through specific mechanisms such as binding to the receptors and damage to the steroidogenic enzymes.

2.1. Estrogenic and antiandrogenic affinity of BPA

Although there are different mechanisms through which endocrine disruptors are able to modify the endocrine response, chemical substances that might simulate the effect of steroid hormones by a reaction with their respective receptors continue to receive considerable attention. The initial step in the mechanism of action of steroid hormones is the binding of the steroid to its receptor or binding protein. BPA has been shown to be able to bind to the oestrogen receptor and initiate transcription of the oestrogen receptor—regulated genes *in vitro*. Several studies using laboratory rodent models have found that defects in the reproductive tract were associated with oestrogenic activity of BPA [21, 22, 33]. The effects of BPA on a wide variety of tissues and cells have, until recently, been thought to be mediated by a single nuclear hormone-receptor oestrogen receptor α (ER α). ER α was consistently demonstrated in rodent Leydig cells. In *in vitro* systems, BPA competes with estradiol for binding with the ER α . After the merger of oestrogen with receptors is the complex transported to the specific site of DNA molecule (oestrogen responsive elements), located in the 5' flanking region. Other components and transcription factors interact with each other and initiate gene transcription [23]. ER α exhibits two distinct gene-transactivating regions, AF1 in the amino terminus and AF2 in the carboxyl terminus [24]. However, the discovery of a second oestrogen receptor β (ER β) has prompted a re-evaluation of the molecular basis for the oestrogen action. Localization of ER β has been variable in the mouse Leydig cells. Structurally, ER β is highly homologous to ER α in the DNA-binding domain (>95% amino acid identity) but shows only a 55% homology in the ligand-binding domain. Furthermore, ER β shows a discrete tissue distribution being the most predominant oestrogen receptor in the ovary, brain and prostate [25]. According to experimental analysis, BPA can produce major alterations in the context of functional gene product synthesis inside the cells with active ER β and major coactivator TIF2, although it can be active in the cells with ER α or ER β and coactivator-1a [26]. However, the validity of these findings has been compromised. BPA is able to induce the expression of the nuclear transcription factor NUR77 in mice Leydig cells, which is involved in LH-mediated testosterone synthesis. After BPA administration, higher activity of protein kinase A and phosphorylation of MAPK mediated with NUR77 expression in Leydig cells was observed. Changes in steroidogenic process within 5 min after administration were detected. This response is too rapid to be mediated by activation of the transcription domains, including classical nuclear oestrogen receptors such as AF1 or AF2. NUR77 mRNA levels were increased above baseline at 1 nm BPA [27]. The oestrogenic activity of BPA was further confirmed by the ability to upregulate the oestrogen target gene expression like pS2 and calbindin-D (9K) in mammalian systems. In fact, BPA is weakly oestrogenic, with a lower potency than the endogenous oestrogen [28, 29]. BPA selectively binds to ER α and ER β and has a higher affinity for ER β in the target cells. It was also found that the binding affinity relative to 17 β -estradiol for BPA at ER β was 6.6-fold higher than ER α . Binding of BPA to the oestrogen receptors alters their ability to recruit coactivators that may be important for the differences in tissue-dependent responses [26]. BPA and other

alkylphenols stimulated the production of a biomarker of oestrogenic activity, vitellogenin, in male fish. Vitellogenin has been used as a biomarker of exposure to antioestrogenic compounds in numerous *in vivo* and *in vitro* studies using fish. It is induced by an oestrogen-dependent activation of gene expression [30]. According to recent molecular studies, BPA is a selective oestrogen receptor modulator, which means that it acts as an oestrogen agonist in some tissues and an oestrogen antagonist in others. Wersinger et al. [31] demonstrated that male mice with deficient in ER α gene were infertile but had a higher serum testosterone levels than their wild-type siblings, indicating that ER α , albeit along with androgen receptors, has a role in mediating the steroid feedback on the pituitary. Compared to researches on the oestrogenic activities of BPA, data on antiandrogenic activities are controversial. Sohoni and Sumpter [32] reported that BPA exhibits antiandrogenic activities, whilst Gaido et al. [33] emphasized that BPA had no antiandrogenic activity. The chemical substances with antiandrogenic properties are able to react with male reproductive function and behaviour by inhibiting the binding of androgens to androgen receptors (AR) and subsequent androgen-induced gene expression. AR is held as an inactive state, being associated with specific 90 kDa heat-shock proteins before exposure to androgens. Upon ligand binding, androgen receptors are translocated into the nucleus and form a complex with specific DNA sequences called androgen-responsive elements (ARE) to enhance the transcription of target genes recruiting coactivators [34, 35]. In this context, we define coactivators as molecules that interact with nuclear receptors and enhance their transactivation. For example, androgen receptor activator-70 (ARA70) was detected in human prostate cells to enhance the androgen receptor transactivation [36]. So, damages of AR gene at molecular level involve the syndrome of androgen insensitivity (AIS) with specific extent of altered reproductive function development, which is associated with defects of androgen receptor-androgen binding, nuclear import, DNA-binding and transcriptional activation. AIS is an archetypal example of a hormone-resistance disorder. In fact, more mutations have been reported in the AR gene than in any other transcription factors [37]. The impairments associated with action of BPA have motivated some researchers to investigate whether BPA could inhibit androgen receptor binding and subsequent androgen receptor-dependent transcription. Once bound to AR, androgen antagonists are imported into the nucleus excluding endogenous androgens from regulating the androgen-dependent transcription. It has been shown that most of the antiandrogenic chemicals contain at least one aromatic ring with a hydroxyl group. The hydroxyl group on the A-phenyl ring of BPA is essential for the inhibitory effect on the AR transactivation. In addition, the hydrophilic substituent at the methylene bridge of BPA is also an important factor for a higher activity [6]. Lee et al. [38] investigated how AR can be affected. BPA inhibits the interaction of AR and its coactivator, interaction of AR and androgens, nuclear translocation of AR and androgen-induced AR transcriptional activity. The inhibition of androgens following BPA treatment is partial and lacks a dose-response relationship, which suggests that the manner of their inhibition may be noncompetitive. On the other hand, according to Sun et al. [39], BPA has the strongest activity to block the gene expression. When they studied the reason why BPA could inhibit the reporter gene expression, it was found that BPA could compete with 5 α -dihydrotestosterone (DHT) to bind to AR. DHT is reduced from testosterone through the action of 5- α -reductase. According to preliminary results, 3,5-substituents of phenol ring of BPA decreased its antiandrogenic activity. Nevertheless 3,5-substituents were reported to

increase the oestrogenic and thyroid activity [40]. A lot of substances with oestrogenic properties are implicated in a number of cancers as an initiator, which confirms carcinogenic character. The potential risk is visible in early life stages related to the development of cancer later in life. The proliferation of human prostate cancer was confirmed at low doses (0.1–10 nM) of BPA mediated with mutation of the AR [4]. It was demonstrated that up to 80% of hormone-refractory tumours are characterized by high production of nuclear AR signifying that the receptor is stimulated even without occurrence of competent ligands. Moreover, amplification of the AR has been reported between 22 and 30% of prostate tumours, providing at least one understanding of how the receptor can be stimulated without occurrence of androgens, such as testosterone and testosterone derivatives [41]. Though in different experimental animal models exposure of low doses of BPA during embryonal stage of development initiated enlargement of prostate size, enhancement of AR expression, reduced differentiation patterns of the prostate and alterations in secretory activity of the gland [42], on the other side, this evidence is questioned by many several experiments that observed no significant impact of this endocrine-disrupting substance in experimental animals [43]. Based on these conclusions, we may speculate that essential compartments of the reproductive system, such as Leydig cells or spermatozoa, are primary targets of BPA. Some experimental studies have shown that BPA affected spermatogenesis probably by competing with testosterone for the cell binding site or other destructive mechanisms. Degeneration of seminiferous tubules and the loss of elongated spermatids were also demonstrated [44]. Furthermore, there are several studies documenting that BPA not only competes with the oestrogen and testosterone receptor, but it also modifies the gene expression of ER α and ER β . Furthermore, BPA has been found to induce cell death by inhibiting the testicular endoplasmic reticulum Ca²⁺ pumps [45]. According to recent information, BPA is able to affect steroidogenesis in Leydig cells not only through receptor-mediated response but also to inhibit the steroidogenic enzymatic activity.

2.2. Interaction of BPA and steroidogenic enzymes

There are mechanisms other than ER- or AR-mediated effects through which BPA could affect physiological functions, including modulation of steroidogenesis and interference with metabolic breakdown of oestrogens and detrimental effects on signalling cascades. Examination of the expression of different steroidogenic enzymes provides information on the molecular basis for alterations in hormone biosynthesis caused by exposure to BPA [46–48]. Essential male reproductive hormones are testosterone and androstenedione. Their biosynthesis is called steroidogenesis where steroidogenic enzymes step out as stable components responsible for specific cascades of reactions which transform cholesterol to endogenous male hormones. Steroidogenic processes start with the transport of cholesterol to the mitochondrial inner membrane where the first steroidogenic enzyme cytochrome P450 cholesterol side chain cleavage enzyme (CYP11A1) uses it as a substrate to produce pregnenolone. Pregnenolone subsequently diffuses to the smooth endoplasmic reticulum, where it is converted to testosterone by the enzymes such as 3 β -hydroxysteroid dehydrogenase, cytochrome P450 17 α -hydroxylase/17,20-lyase (P450c17) and 17 β -hydroxysteroid dehydrogenase (17 β -HSD). The first reaction in the smooth endoplasmic reticulum is catalyzed by 3 β -HSD to progesterone. P450c17 catalyses two reactions that convert progesterone to 17 α -hydroxyprogesterone

and then to androstenedione. 17 β -hydroxysteroid dehydrogenase catalyses the last step from androstenedione to testosterone.

Recent experimental studies have demonstrated that the production of both androstenedione and testosterone was inhibited by BPA in a concentration-dependent manner over the course of 24 h incubation. Lower concentrations of androstenedione and its direct downstream product, testosterone, after the exposure to BPA are consistent with a direct inhibition of enzymatic activities, such as 3 β -HSD, cytochrome P450c17 and 17 β -HSD. A decrease in the activity of 17 α -hydroxylase resulted in a lower production of its direct product 17 α -hydroxyprogesterone. Moreover, the decreased activity of 17,20-lyase inhibited the rate of 17 α -hydroxyprogesterone conversion to androstenedione, which led to in a 7.7-fold reduction in the androstenedione synthesis and a 2.4-fold reduced testosterone level [49]. It has been reported that prenatal exposure of BPA in rodents causes a reduction in the testosterone production. It is possibly caused by the downregulation of the steroidogenic enzymes in the Leydig cells and an inhibition of LH secretion [50]. Ye et al. [51] confirmed a dose-dependent inhibition of human 3 β -HSD and P450c17 by BPA. At 10 μ M, BPA also weakly but significantly inhibited human and rat 17 β -HSD activities. In general, human steroidogenic enzymes are more sensitive to BPA than rat enzymes. The results also demonstrate that BPA partially competes with cofactor NAD⁺ (for 3 β -HSD) in the cofactor binding site of this enzyme. The second essential enzyme 17 β -HSD, which is responsible for testosterone synthesis from androstenedione, was observed to decrease the activity of this enzyme. 17 β -HSD accounts for most of the circulatory testosterone in males and in the case of genetic mutation induced by BPA may cause the autosomal recessive genetic disorder male pseudohermaphroditism in which males often are born with female external genitalia and without a prostate [52, 53]. The aromatase enzyme, which is encoded by the CYP19 gene and catalyses the conversion of androgens to oestrogens, is expressed more in the male reproductive tract than in other tissues in rodents. Some experimental data show that BPA caused a direct inhibition of aromatase gene expression and oestrogen biosynthesis. Disruption of CYP19 gene expression of aromatase in Leydig cells was ER α mediated as oestrogenic agents act via ER α to upregulate the promoter region of the aromatase gene [54, 55]. Some experimental studies indicate that a decreased androgen production by Leydig cells does not correlate with level of testosterone in serum after chronic BPA exposure which can be due to compensatory mechanisms initiated *in vivo*, for example, elevated pituitary luteinizing hormone. Higher serum luteinizing hormone levels in BPA-treated rats presumably resulted from a decreased sensitivity to the androgen negative feedback on the hypothalamus and pituitary and the consequent stimulation of luteinizing hormone secretion [25]. Nikula et al. [56] demonstrated that exposure to environmentally relevant BPA levels had also adverse effects on testicular function by decreasing pituitary LH secretion and reducing Leydig cell steroidogenesis. For example, exposure of pubertal rats to 2.4 μ g/kg for 15 days indicated a decreased testosterone levels as well as Leydig cell androgen biosynthetic capacity. On the other hand, small but significant reduction in LH was observed in rats treated with BPA after 2 weeks of treatment, although this effect had disappeared after 5 weeks. Even if BPA inhibits production of testosterone through reduced secretion of LH, it is proved that BPA binds with LH receptor ligand binding, and releasing of LH from the receptor may cause reduction of steroidogenic activity. In the human

testes, isoforms of ER β implying that BPA is able to modulate oestrogen synthesis have been localized. Imbalance in the androgen and oestrogen action, during early stages of differentiation, may induce potential damage of male reproductive parameters and sexual behaviour in adulthood [57]. Both oestrogen and testosterone are necessary for development and functions of male reproductive system tissues; inhibition of steroid hormone biosynthesis can be associated with abnormalities of testes after BPA exposure.

2.3. Effects of BPA exposure during gestation through puberty

We recognize that the development is epigenetic, which refers to changes in gene activity during developments that are mediated by chemical signals. Autocrine, paracrine (growth factors) and endocrine (steroids) signals coordinate the direction of tissue differentiation during critical periods in development. Androgens, mediated by the AR, do play an indispensable role in induction of male sex differentiation and development of the male phenotype. It has been demonstrated that the developing embryo may be much more susceptible to harmful effects of environmental contaminants than adult animals. A high *in vivo* BPA efficiency during embryonic development is caused by low BPA binding to oestrogen-binding proteins in plasma that are intended for control of endogenous estradiol absorption into cells and by the low embryos and newborn capacity to metabolize and inactivate BPA in the liver [58]. Potential BPA cumulation in embryos is supported by findings which demonstrate that BPA status in amniotic fluid at 15–18 weeks of pregnancy is fivefold higher than BPA status in serum of pregnant and non-pregnant females [59]. After treatment of mice during gestation with BPA in dose 100 mg/kg BPA (given subcutaneously), BPA was identified in the brain, liver, foetal sera, uterus and testes 30 min after exposure [60]. Significant effects caused in rats and mice by exposure during development to doses of BPA involve structural and neurochemical changes throughout the brain associated with behavioural changes, such as hyperactivity, learning deficits and increased aggression. Increased aggressiveness in male CD-1 mouse offspring occurred as a result of oral administration of 2 μ g/kg/day of BPA to pregnant females [61]. A lot of current researches have observed that BPA treatment of rodents during gestation even in low doses induced persistent impact on tissues of male reproductive organs and female descendants. Recent experiments by Nagel et al. [62] and Vom Saal et al. [63] reported that administration of low oral doses of BPA (2–20 μ g/kg/day) to pregnant female mice produced statistically significant increase in the weights of the prostate and decrease in epididymis weights and the efficiency of sperm production in their male offspring. These findings are important for several reasons. Firstly, they provide evidence that microgram volumes of BPA are able to cause teratogenic and genotoxic effects in foetus during gestation. Secondly, they prove that BPA is both absorbed and active after oral administration. When female mice were administered to BPA, in the testicles of BPA-exposed male offspring, expression of anti-Müllerian hormone and steroidogenic acute regulatory protein (StAR) was inhibited, and also size of testicles was reduced. In addition, negative impact was persisting in the sexually developed male offspring at 42 postnatal days [64]. From the viewpoint of organ systems and organisms, sexual steroid hormones are responsible for different implications of male fertility development, such as development and keeping of sexual organ system and secondary sex differences. One of the most interesting findings concerning organizational

effects of BPA is decrement in sex differences that are usually evident between sexes. Fascinating is also the effect of this substance only on one of the sexes. The reactions causing diverse impact of BPA within genders are still not fully understood, but there is known mechanism that metabolism of endocrine disruptors, such as BPA, is under the influence of testosterone, whilst BPA is able to alter testosterone metabolism [50, 65]. It follows that particular impact of BPA on male sex in the reproductive organs and tissues is caused by cross-reactions with sex steroid hormones. In pregnant female rats exposed to BPA, serum testosterone levels were decreased in male foetus and pups. The testosterone inhibition is probably induced by BPA-suppressive effect on testicular Leydig cells. In fact, BPA inhibits expression of StAR protein, 17 β -HSD and others. Protein expression of luteinizing hormone receptor is also compromised following BPA exposure and may lead to decreasing androgen biosynthesis [66, 67]. Direct mitogenic effect of BPA on the foetal prostate has been demonstrated in some experimental studies. Prostate ductal budding begins in mice 2 days before birth. Prostate development is dependent on DHT production. AR expression in the prostatic mesenchyme is required for directing growth and branching morphogenesis of epithelial buds, presumably by induction of paracrine factors secreted by the mesenchyme [68, 69]. There is evidence that oestrogens modulate the activity of androgens in regulating prostate development. The mesenchyme in mice and rats responds to oestrogens via ER α whereas in the human prostate ER β . Prostatic growth and androgen receptor ligand-binding activity are permanently decreased in response to high doses of BPA during development [70]. Study of Alonso-Magdalena et al. [71] showed that BPA in small doses throughout sensitive stages of development is able to induce negative effect on glucose homeostasis and insulin sensibility. In this experiment mice exposed to BPA by orally administration were pregnant. These mice exhibited glucose intolerance and increased insulin, glycerol, triglycerides and leptin status in plasma compared to control group of pregnant mice. Currently, there is a lot of evidence that this xenobiotic has severe impact not only on mammals but also on amphibian. *Xenopus laevis* exposed to BPA (10^{-7} mol/L) showed feminization of male sexual characteristics, whereas this impact was significant in female larvae phenotypes compared with control individuals [72]. Primary sexual differentiation in *X. laevis* tadpoles is initiated with an indifferent gonadal phase before differentiation into ovaries or testes occurs. It has been shown that the most sensitive period for the induction of sex reversal in *X. laevis* is between stages 50 and 52 [73]. This sex reversal phenomenon was discovered before, when *X. laevis* tadpoles were treated with 17 β -estradiol during gonadal differentiation, and observed that all treated tadpoles developed as fertile females. Exposure to exogenous sex steroids and structurally related substances like BPA, during sensitive phase of sexual differentiation, alters the genetically determined gender of the animals [72, 74]. It is suggested that although the definite fate of the primordial germ cells is determined by genetic factors, alterations in the sex steroid hormonal milieu can override this genetic mechanism. Recently, attention has been drawn to reports of BPA-induced gonadal malformation in either testes or ovaries. In the context of feminization impact, reproductive dysfunction is associated with the development of ovo-testis condition. This phenomenon is characterized as the presence of eggs in the testicular tissue. Ovo-testis structure was observed in some gonochoristic fish species (*Oryzias latipes*) both in laboratory and wild animals exposed to BPA. The induction of ovo-testis was observed in *O. latipes* after 60-day posthatch only in the 1820 μ g/L treatment. Growth suppression was also observed in

concentration-dependent manner. This suppression might be caused by oestrogenic and alkylphenolic character of BPA [75]. Ashfield et al. [76] suggested that the growth suppression of rainbow trout exposed to alkylphenol chemicals might be influenced by the oestrogenic activity of these chemicals in vitellogenin synthesis, which diverts energy resources from growth. The oestrogenicity of BPA can also prevent anti-Müllerian action on the Müllerian ducts in the male, leading to the feminization of male foetus, and feminization can be initiated via upregulation of genes necessary for normal differentiation of ovary tissues (Foxl2 and Wnt4), with simultaneous inhibition of genes required for testis differentiation (Sox9 and Fgf9) in the foetus [77, 78]. Experimental studies in rodents suggest that BPA causes reproductive toxicity that persists into the second generation. Experimental study of CD-1 mice revealed that exposure to high levels of BPA via ingestion caused a longer gestation period and decreased litter size in the high-dose range. The first female generation appeared to be the most affected as they delivered 51% fewer pups when mated with control partners. The males sired 25% fewer pups in the high BPA group [79].

3. The potential impact of BPA on the spermatogenesis

Spermatogenesis is under the control of the hypothalamic-pituitary-testicular axis and the thyroid gland. Dysfunction of this axis, initiated by endocrine disruptors such as BPA, may result in a discontinuance or alteration of spermatogenesis [80]. BPA acts through sex steroid-mediated hormone cascade pathway to influence functions of reproductive system, and it is likely that BPA is also able to modulate specific characteristics of sexually dimorphic systems, in particular gender differences in the mental functions and behaviours of the sexes [81]. The harmful impact of BPA on male reproductive function may occur over embryonic, pubertal and/or adult life [80]. Many current studies have demonstrated that low doses of this widespread oestrogenic chemical substance can induce strong, membrane-initiated oestrogenic effects [82], indicating that low levels of BPA exposure might interfere with normal oestrogenic signalling pathway [4]. It is known that oestrogen receptors are expressed in the Leydig cells (ER α), whereas ER β have been described in Sertoli cells, pachytene spermatocytes and round spermatidis of the adult rat and male testis. ER has been also shown to be expressed in other tissues of the male reproductive tract [83]. Recent *in vitro* study has shown that unconjugated (aglycone) BPA binds to ER α and β , generating weak oestrogenic action, and has also high affinity for two membrane-bound oestrogen receptors, G-protein-coupled oestrogen receptor 30 and membrane oestrogen receptor α , in addition to orphan nuclear oestrogen-related receptor γ [84]. Due to previous evidence about biological activity of BPA, in which BPA has the ability to induce division of cultured human breast cancer cells and bind with the ERs, one cannot rule out the possibility that BPA is able to impact process of spermatogenesis in males [83]. Another relevant action by which endocrine disruptors perform their negative impacts on male sexual system is to break the balance between oxidants and antioxidants in testicular tissues, which is associated with the development of oxidative stress and consequent harmful effect on spermatogenesis [85]. The activity of superoxide dismutase, catalase

and glutathione peroxidase was reduced, whilst the status of peroxide and peroxidation of lipids significantly increased in the rats exposed to BPA compared to control animals without BPA treatment. Data obtained in this experiment showed that upgrade levels of BPA induce fall of antioxidant defence system and cause oxidative stress in rat epididymal sperm [8]. Also, BPA is administrated during the embryonic/foetal life and over infancy via the placenta and milk; reactive oxygen species were induced in mice testis. Peroxidation of the testis was enhanced and finally resulted in their underdevelopment [86]. *In vitro* studies demonstrated that BPA could cause oxidative stress in sperm cells, which leads to accumulation of free radicals, together with the reduction of cell antioxidant system activity. These oxidative responses were associated with spermatozoa quality decrease, as measured by decline in the rates of spermatozoa motility and velocity. BPA administration also leads to depletion of ATP metabolism and significant DNA fragmentation in sperm cells [87]. Besides that, D'Cruz et al. [88] study suggested that the oestrogenic and oxidative stress-inducing ability of BPA could have supported to the violation of glucose homeostasis in the testis. Results show that sustained exposure to BPA may damage the testicular functions by targeting the glucose metabolism in the testis.

3.1. Spermatogenesis and sperm function affected by BPA

A lot of experiments with BPA have confirmed that this chemical substance, even at levels under doses that are considered as safe for human population, is able to impair sexual functions and behaviour in rodents [89], and for male reproduction, it is proved that the exposure of adult rats to environmental doses of BPA can reduce activity of spermatogenesis and sperm count [8, 83]. *In vivo* study with adult rats suggests that low dosage (2 µg/kg body weight) of BPA after oral administration impairs spermatogenesis by reducing reproductive hormones to stop meiosis of germ cells and activating the Fas/FasL pathway to induce the apoptosis of germ cells, lowers the biosynthesis and secretion of testosterone via inhibiting the activity of GnRH neurons, and decreases the expression of steroidogenic enzymes. Subsequently, declining testosterone rate was accompanied by reduction of sperm concentration [90]. Another recent *in vivo* study with experimental rats evaluated the potential impact of BPA in the doses of 1, 5, 10 and 100 mg/kg body weight on spermatogenesis. Seminiferous tubules were devoid of spermatozoa or were filled with immature germ cells and cellular debris with sloughing of germ cells into the seminiferous tubular lumen. Furthermore, the seminiferous epithelium appeared to be disintegrating with loosening of the intercellular bridges between germ cells as well as between germ cells and sertoli cells. Epididymal tubules also showed empty lumen or lumen filled with cellular debris [91]. Study of Furuya et al. [92] refers to stunted development of testicular tissue in male chickens that were treated for up to 23 weeks by oral administration of BPA in doses low as 2 mg/1000 g body weight every 2 days; chickens receiving BPA showed reduced weight of testes, with the smaller seminiferous tubules exhibiting constrained spermatogenesis. The antispermatogenic potency of BPA proved in experimental animals has been confirmed by several epidemiological studies realized among groups of exposed human males. Epidemiological evidence in humans indicates that urine

BPA levels are highly associated with decline sperm concentrations, indicating an essential association between BPA exposure and production of sperm cells. Compared with men who did not have noticeable urine BPA levels, those with noticeable urine BPA had more than three times the chance of lowered sperm concentration and lower viability of sperm, but there was no found correlation between urine BPA levels and semen volume or sperm morphology [93]. Wang et al. [94] reported an implication between higher urinary BPA concentrations and clinically abnormal thyroid hormones (elevated serum-free T3 levels) that also affect spermatogenesis. Direct action of BPA on spermatozoa is still unclear. Human sperm incubated with 1 μ M of BPA showed no significant changes in influxes of calcium and pathological acrosome reaction in the sperm cells. Equally, the genetic material of sperm cells, as evaluated by the TUNEL protocol, comet and redox activity were not damaged by *in vitro* BPA exposure [95]. These facts indicate that the severe BPA impact on male sexual system is caused *in vivo* by different mechanisms, in the concrete alterations in the function of the hypothalamic-pituitary-gonadal axis and thyroid hormone activity [50]. Motility is still the most important parameter in the semen analysis and initial investigation of the male fertility factor, and it is a basic prerequisite that enables sperm cells to fertilize the egg cell [96]. Spermatozoa motility closely relates with mitochondrial activity, because spermatozoa contain many mitochondria helically arranged around the middle piece axonema. Mitochondria play a key role in the energy production through the generation of adenosine triphosphate and maintenance motility of spermatozoa [97]. Possibly, BPA uses endogenous oestrogen signalling pathway and similarly to 17 β -estradiol modulates sperm motility. This may be related to its effects on sperm mitochondrial potential and, thus, the generation of ATP. It appears because of the proven middle piece localization of ERs in sperm. This region of sperm cell is characteristic for mitochondrial incidence, and mitochondria are possibly target organelles for oestrogen action [98]. In addition, there is proof that mitochondrial enzymes in the testis such as succinate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, monoamine oxidase and NAD dehydrogenase decreased after BPA exposure [99]. Adult male mice show significant reductions in testicular sperm counts, as well as in epididymal sperm counts, after exposure to 25 ng/kg body weight of BPA [100]. Similarly, a reduction of sperm count and motility and an increase of sperm morphological abnormalities, following 2 weeks of BPA administration (10–40 mg/kg body weight), were found [101]. A dose-dependent effect of BPA on male birds was observed also in Singh et al. [102] *in vivo* experiment with physical attributes of semen such as semen volume, sperm motility and sperm concentration. Semen volume was highest in low dose (1 mg/kg body weight), whilst sperm concentration was lowest, indicating tail off of bird semen treated with low-dose BPA. The sperm motility was found smaller in high dose (5 mg/kg body weight) of BPA. It is supposed that high dosage of BPA rises the availability of metabolically active BPA in the blood, which is associated with lower motility parameters of sperm due to blockage of the Ca²⁺ channels activated by voltage or by rising oxidative stress in the sperm cells [103]. The results obtained from *in vitro* study of Lukacova et al. [104] confirm that BPA have the detrimental effect on bovine spermatozoa motility. This study also showed that BPA in different doses (1, 10, 100 and 200 μ g/mL) is able to decline mitochondrial activity and spermatozoa viability and caused mitochondrial dysfunction by the increasing intracellular formation of superoxide radical. Analysis of sperm motility parameters confirmed the significant differences between the experimental samples from groups with doses of BPA higher than 100 μ g/mL and the control samples. Sperm motility

results obtained after 6 h of cultivation at the doses higher than 10 µg/mL of BPA showed significant reduction of motility, and after 24 h cultivation, it was found that the doses less than 10 µg/mL statistically improve motility parameters, whilst the doses higher than 100 µg/mL significantly reduced motility in comparison to samples from control group. Results of motility in this study correlate with other *in vitro* experiments with sperm of various species. Motility parameters of experimental mice sperm were measured following 6h of *in vitro* cultivation with increasing BPA doses (0.0001, 0.01, 1 and 100 µM). Number of motile sperm cells was significantly reduced after incubation with BPA in concentration of 100 µM [105]. Also, another study with chicken sperm showed that environmentally serious concentration of BPA (0.74 mM) significantly decreased motility as well as fertilizing ability, live sperm percentage and mitochondrial membrane potential [102]. Exposure to various BPA concentrations (0.6, 4.5 and 11.0 µg/L) negatively affected motility of fish too [106]. Study with human male observed a demonstrable correlation, although not statistically significant, between BPA exposure, specifically urinary BPA concentration, and altered sperm parameters, such as reduced sperm count affected sperm motility attributes and morphology, and increased damage of DNA integrity in sperm, between infertile men [107]. According to Danish research, 98% of tested men had quantifiable rates of BPA after measuring in urine with an average amount of 3.25 ng/mL. Urine samples from group of tested men with high amounts of BPA also showed considerably higher testosterone, estradiol and luteinizing hormone status than urine samples with lowest amount of BPA. These men showed loss of spermatozoa motility too [108].

BPA experiments on different animals exhibit that impact is usually more damaging throughout in utero stage, which is the most sensitive developmental phase for the foetus. It has been observed that this chemical substance is able to generate different injuries in the foetus, including male embryos feminization, reduced function of the testes and epididymides with breakdown of tissues, enlarged prostate, shortening of anogenital distance and alteration of adult sperm parameters, such as sperm count, motility and density. BPA is also able to affect embryo thyroid development [80]. Recent findings support another additional BPA activity mechanism, by a non-genomic pathway, initiated at membrane receptors, including standard ERs and/or G-protein-coupled receptor 30 [109]. By disrupting levels of hormone or receptor activity, the negative effect of this chemical substance may be to modulate male reproductive organ development throughout foetal life. In addition, harmful BPA impact can be more noticeable and nonreversible throughout this phase of development, in contrast to adults, who reached a functional sex maturity and physiology, in which the harmful impact is eventually not persistent since the first exposure [110]. In utero exposure to BPA was found to cause negative effects on reproductive organs in rodents. In utero exposure of pregnant CD-1 mice to BPA in amount 50 µg BPA/kg body weight/day during 16–18 days of gestation showed increasing the anogenital distance in male young [111]. This conflicts with study of Chahoud et al. [112], who presented shortening of the anogenital distance, following prenatal BPA exposure. However, these studies exhibited that BPA has the ability to alternate anogenital distance during prenatal life. Alteration in the development and tissue organization, changes in prostate gland weight, reduced sperm efficiency and daily sperm production were also observed [58, 62]. Oral administration of 2–20 ng BPA/g body weight to female mice on 11–17 gestational days exhibited significant decline of relative testis weight of male

young [61]. Vom Saal et al. [63] researched BPA exposure on male mice during pregnancy and observed raising size for preputial glands and reduced size of epididymides, as well as reduced capacity of daily sperm production. When female mice were co-administered with BPA in combination with di(2-ethylhexyl) phthalate, another chemical plastic substance, the expression level of anti-Müllerian hormone was decreased in the testicular tissue of treated young males and also reduced the size of testes. And more significantly, the negative impact was sustained in the sexually mature young at postnatal day 42, associated with decrease counts of epididymal sperm cells [64]. A decline in fertility, daily sperm production and count and motility of sperm in BPA-exposed male offspring over maturity was also reported in Salian et al. study [113].

3.2. Impact of BPA on Sertoli cell function

Normal function of Sertoli cells that are part of a seminiferous tubule is crucial in the spermatogenesis. Process of differentiation and production of mature sperm cells is under the control of the FSH because Sertoli cells are equipped with FSH receptors on their membranes and are activated by secretion of this adenohypophysis hormone. Inhibition of the Sertoli cell function by BPA, directly or indirectly through reduction of hormone synthesis, may impair reproductive function in exposed males [80]. Sertoli cell function is to provide support, in other words, provide the adequate metabolic and structural background for developing spermatozoa because a lot of factors important for gamete maturation are associated with functions of somatic Sertoli cells. Consequently, any agent that impairs the viability and the function of Sertoli cells may have profound effects on spermatogenesis [114]. Experimental study dealing with impact of BPA on Sertoli cells demonstrates that exposure of cultured Sertoli cells to BPA decreased cell viability. Treated cells showed alterations in morphology, including blebs on membrane, breakdown of cytoskeletal structures, cell rounding and condensation and fragmentation of DNA that conform to the morphological changes of apoptosis. Results strongly suggest that death of BPA-exposed Sertoli cells is not due to necrosis, but to activation of the apoptotic signal pathways in the cells [115]. In cultured Sertoli cells, BPA also has been shown to induce apoptosis. Moreover, BPA-induced damage of Sertoli cells has been reported by blocking endoplasmic reticulum Ca^{2+} homeostasis [116] and the ectoplasmic specialization between Sertoli cells and spermatids [117]. Previous findings suggested this chemical inhibits endoplasmic reticulum Ca^{2+} -ATPase activity and mobilizes intracellular Ca^{2+} concentration in mouse Sertoli cell lines, TM4 [45]. Fiorini et al. [118] also studied mechanism of BPA action on Sertoli cells. Sertoli cells establish intercellular junctions that are essential for spermatogenesis. Currently, it is known that SerW3 Sertoli cells form characteristic protein elements of cell junctions such as gap junctions with connexin 43, tight junctions with occludin and zonula occludens-1 and anchoring junctions with N-cadherin. This xenobiotic substance impairs junctions between adjacent cells in the tissue by decreasing their number or by inducing abnormal position of these membrane proteins within cells. In addition, BPA is also able to induce downregulation of several genes associated with Sertoli cell function (Msi1h, Ncoa1, Nid1, Hspb2 and Gata6) in 6-week-old-male mice after prenatal exposure [119], thereby disrupting the blood-testis barrier and impairing spermatogenesis [120].

3.3. Induction of oxidative stress by BPA in the male reproductive system

Exposure to environmental toxicants such as BPA induces the overproduction of reactive oxygen species, leading to testicular oxidative stress. It is known that BPA decreases the activity of the male-specific cytochrome P450 isoforms, and cytochrome P450 has been shown to induce reactive oxygen species that impairs sperm functions and spermatogenesis [121]. Reactive oxygen species can modify the sperm cytoskeletal and axoneme structures, causing a decrease of sperm motility parameters and low probability of sperm-oocyte fusion and therefore leading to low fertility potential [122]. Free radicals are also able to impair the genetic information within the nucleus of the sperm cell, and this damage to the genome may be translated into infertility [102]. In El-Beshbishy et al. [123] experiment, body weight of BPA orally applied for 14 days to male rats was 10 mg/kg, and considerable decline of enzymes with antioxidant activity in testicular tissue such as catalase, glutathione reductase, superoxide dismutase and glutathione peroxidase has been found. Also, hydrogen peroxide quantity and lipid peroxidation were increased in testes and spermatozoa of BPA-treated animals. Kabuto et al. [86] investigated the modifications in endogenous antioxidant capacity and oxidative damage in the mice testis exposed to BPA, whilst animals were treated with BPA during embryonic and foetal phase of life and during lactation phase by oral administration of drinking water with BPA (5 or 10 µg/L) to their pregnant/lactating mothers and male mice were killed in the fourth week of life. BPA increased levels of thiobarbituric acid-reactive substances in the testis, and results suggested that exposure to this chemical substance induces tissue oxidative stress and peroxidation, ultimately leading to testicular underdevelopment. In another *in vivo* study, testicular antioxidant enzymes were impaired by a very low dose (0.005 mg/kg body weight/day) of BPA following 45 days of exposure [124]. Exposure to BPA may also decline antioxidant enzyme activities and induced lipid peroxidation in both epididymides and sperm cells in rats after administration of BPA (0.2, 2 and 20 µg/kg body weight per day) for 45 days and resulted in inhibition of epididymal motility of sperm and number of sperm depending on the level of dose in treated rats as compared with the corresponding group of control animals [8]. Hulak et al. [87] reported that BPA exposure to fish sperm at concentrations 1.75–10 µg/L is capable of inducing oxidative stress, leading to impaired sperm quality, DNA fragmentation and intracellular adenosine triphosphate content. In research with human spermatozoa, *in vitro* exposure to BPA at concentration starting from 300 µM equally could affect sperm integrity through the induction of prooxidative as well as apoptotic mitochondrial dysfunction. It was associated with an increased mitochondrial generation of superoxide anion, caspase-3 and caspase-9 activation and motility decline [125]. Similar results also provided Lukacova et al. [104] in an *in vitro* study with bovine spermatozoa. Generation of superoxide radical within sperm cells was measured by the NBT assay after 24h incubation with BPA. The results showed that in samples of experimental groups, the quantity of superoxide radical increased unlike to samples in control group without BPA. At the dosages higher than 100 µg/mL of BPA, significant differences were noticed. The viability of spermatozoa in metabolic activity assay (MTT) declines in all experimental groups, but significant differences were observed only at the highest doses of BPA after 24h of *in vitro* cultivation. These results demonstrated that BPA can directly promote biological damage by oxidative stress and induces

apoptosis in sperm cells across a range of animal species, including humans. The potential impact of BPA on essential reproductive parameters is presented in **Figure 1**.

3.4. Epigenetic effect of BPA on male reproduction

Some chemicals with oestrogenic properties pass through CYP-mediated redox cycle to quinones. Quinones represent biologically active molecules that can bind by covalent bonds to DNA and proteins occurring in the nucleus, such as DNA and RNA polymerases. *In vitro* study of Atkinson and Roy [126] showed that BPA is oxidized by 70% to bisphenol o-quinone. Authors also postulate that BPA is oxidized first to semiquinone and after that oxidized to bisphenol o-quinone. The chemical reaction of DNA with bisphenol o-quinone produced 6–8 adducts. Quinones and several other reactive components have short half-lives; therefore, negative impact of BPA arises especially in oestrogen-sensitive structures. Given that it is expected that modification to quinones and generating of DNA adducts intervene in structures that binding and retaining BPA. Formation of DNA adducts in tissues of sexual system throughout organogenesis can cause genetical imbalance, gene modifications and chromosomal mutations with permanent effects for mature individuals [127]. Whether irreversible binding of BPS to DNA through metabolic activation may be responsible for some of the toxic effects produced by BPA is not clear. Atkinson and Roy [128] also present an *in vivo* study, where binding BPA to DNA was confirmed. BPA is first converted to hydroxylated BPA. Like catechol BPA then enters into redox reactions. During this redox cycling, BPA is enzymatically oxidized first to semiquinone. Bisphenol semiquinone is then further oxidized to bisphenol-o-quinone. BPA without metabolic activation did not bind to DNA [129]. In con-

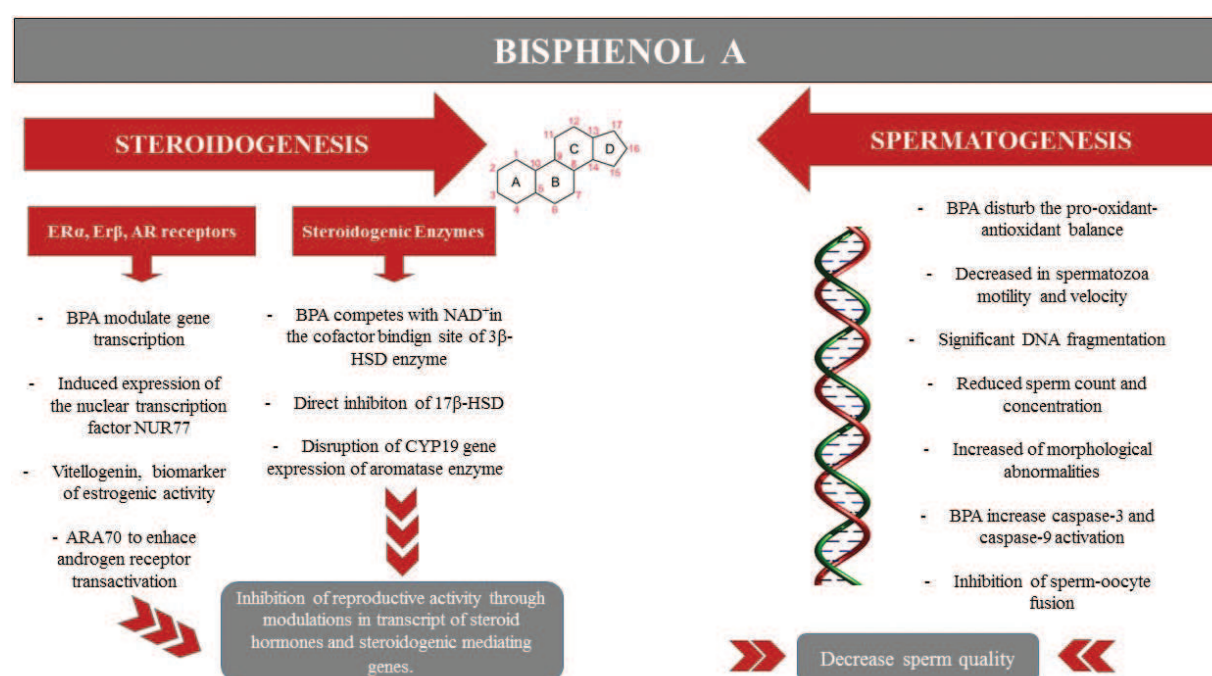


Figure 1. A model summary for the effects of bisphenol A (BPA) on reproductive system. NAD⁺, nicotinamide adenine dinucleotide; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase.

clusion, directly after metabolic change to metabolites with reactive properties, DNA with covalently converted nucleotides leads to mutational alterations and, therefore, can represent a key attribute in cellular toxicity and development of tumorigenesis process.

Experiments on toxicity of reproductive system exhibited that pregnant female exposed to BPA in prenatal period involved significant fertility disorders of not explicitly F1 male descendants but also subsequent F2 and F3 generations. It also causes increased occurrence of damage during implantation phase in all the three generations. This increase was significant in F3 generation suggesting that this xenobiotic is able to perform its impacts through male germline [130]. Current studies have also begun to suggest the possibility of translation of early exposures to physiological modifications later in life and across generations by epigenetic mechanisms such as methylation-mediated promoter silencing [4]. Epigenetics deals with molecular processes that are associated with hereditary and permanent changes in gene expression. However, these changes do not involve modifications in sequences of DNA. DNA sequences stay constant, but the expression or silencing of genes and regions of gene is mediated by different epigenetic processes, such as methylation, and in reaction to different exposures of environment. DNA methylation is a process by which methyl groups are linked to the DNA molecule, specifically to the cytosine in cytosine-phosphate-guanine segment of DNA. Methylation is able to modify the activity of a DNA sequence without modifying the sequence, and it may cause silencing of gene expression in the segment of DNA [131]. Experiments with rats have demonstrated that BPA exposure and its impact on sexual hormones may cause persistent alterations in the whole male hypothalamic-pituitary-gonadal axis, including development of transgenerational alterations in the levels of steroid hormone receptors in testes, motility of spermatozoa as well as sperm count [113]. Adverse effect of BPA on male germ cells is not matter only prenatal exposure; Tiwari and Vanage [130] experiment demonstrated that adult male rats exposed to 5 mg/kg body weight of BPA during a time of 6 days will generate fatal mutations in spermatozoa. It leads to low sperm motility parameters and sperm production. Due to these facts, it is key to research the epigenetic alterations in male fertility caused by BPA also in later life, not only in critical stages of development. BPA exposure has also been connected to sexually dimorphic alterations in anxiety-like behaviour and general motor activity [132]. This suggests that dose-dependent effects of BPA on emotional aspects and sexually dimorphic manner are associated with demasculinization of characteristic male behaviour [133]. There were also observed alterations in sexual behaviour, especially in a decreased performance in latency and frequency of intromission among BPA-exposed rodents [10, 11]. An impairment in the timing of copulatory sequence was found in Sprague-Dawley male rats, perinatally exposed to BPA via oral administration during pregnancy or lactation [11] or exposed throughout early stages of development [134]. In animals that were postnatally treated by oral administration of BPA, a decrease activity in terms of latence and intromission frequency was noticed [11]. Moreover, same effect in this direction was observed with animals treated in phase of early puberty [134]. Results obtained from study with workers of manufacturers of BPA in China also showed relevant proof that BPA exposure at work significantly increases the possibility of sexual dysfunction in male. The results were the same for all tested parameters that were measured regarding to male sexual dysfunction, all indicating increased risks associ-

ated with exposure of BPA. The noticed findings remained after monitoring of wide physiological and psychological aspects that may be related to reproductive disorders between workers exposed to BPA and unexposed workers. Moreover, the relationship between dose and response for found associations also supports the discovery. These findings strengthen probably essential association between exposure to high doses of BPA and raising possibility of reproductive dysfunction in males [93]. Apoptosis of spermatogenic cells was also affected intergenerationally with differential DNA methylation of sperm promoter regions in the F3 generation which was observed in all exposed male lines [135]. Considering the imprinted-like nature of the modified epigenetic DNA methylation sites, sperm cells transfer this epigenome and adult onset disease phenotype to next generations, which is termed epigenetic transgenerational inheritance [136].

Acknowledgements

This study was supported by the Slovak Research and Development Agency Grant nos. APVV-15-0543 and APVV-15-0544.

Author details

Tomáš Jambor, Bistáková Jana, Greifová Hana, Tvrdá Eva and Lukáč Norbert*

*Address all correspondence to: norolukac@gmail.com

Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Nitra, Slovak Republic

References

- [1] Junk GA, Svec HJ, Vick RD, Avery MJ. Contamination of water by synthetic polymer tubes. *Environmental Science and Technology*. 1974;**8**:1100-1106. DOI: 10.1021/es60098a009
- [2] Vitku J, Sosvorova L, Chlupacova T, Hampl R, Hill M, Sobotka V, Heracek J, Bicikova M, Starka L. Differences in bisphenol A and estrogen levels in the plasma and seminal plasma of men with different degrees of infertility. *Physiological Research*. 2015;**64**:303–311
- [3] Pavlovich CP, King P, Goldstein M, Schlegel PN. Evidence of a treatable endocrinopathy in infertile men. *The Journal of Urology*. 2001;**165**:837–841. DOI: 10.1016/s0022-5347(05)66540-8
- [4] Wetherill YB, Akingbemi BT, Kanno J, McLachlan JA, Nadaf A, Sonnenschein C, Watson CS, Zoeller RT, Belcher SM. *In vitro* molecular mechanisms of bisphenol A action. *Reproductive Toxicology*. 2007;**24**:178–198. DOI: 10.1016/j.reprotox.2007.05.010

- [5] Nakamura D, Yanagiba Y, Duan Z, Ito Y, Okamura A, Asaeda N, Tagawa Y, Li C, Taya K, Zhang SY, Naito H, Ramdhan DH, Kamijima M, Nakajima T. Bisphenol A may cause testosterone reduction by adversely affecting both testis and pituitary systems similar to estradiol. *Toxicology Letters*. 2010;**194**:16–25. DOI: 10.1016/j.toxlet.2010.02.002
- [6] Xu LC, Sun H, Chen JF, Bian Q, Qian J, Song L, Wang XR. Evaluation of androgen receptor transcriptional activities of bisphenol A, octylphenol and nonylphenol *in vitro*. *Toxicology*. 2005;**216**:197–203. DOI: 10.1016/j.tox.2005.08.006
- [7] Herath CB, Jin W, Watanabe G, Arai K, Suzuki AK, Taya K. Adverse effects of environmental toxicants, octylphenol and bisphenol A, on male reproductive functions in pubertal rats. *Endocrine*. 2004;**25**:163–172. DOI: 10.1385/endo:25:2:163
- [8] Chitra KC, Latchoumycandane C, Mathur PP. Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. *Toxicology*. 2003;**185**:119–127. DOI: 10.1016/s0300-483x(02)00597-8
- [9] Welshons WV, Nagel SC, Vom Saal FS. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. *Endocrinology*. 2006;**147**:56–69. DOI: 10.1210/en.2005-1159
- [10] Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenbergh JG, Walser-Kuntz DR, Vom Saal FS. *In vivo* effects of bisphenol A in laboratory rodent studies. *Reproductive Toxicology*. 2007;**24**:199–224. DOI: 10.1016/j.reprotox.2007.06.004
- [11] Farabollini F, Porrini S, Della Seta D, Bianchi F, Dessi-Fulgheri F. Effects of perinatal exposure to bisphenol A on sociosexual behavior of female and male rats. *Environmental Health Perspectives*. 2002;**110**:409–414. DOI: 10.1289/ehp.02110s3409
- [12] Li D, Zhou Z, Qing D, He Y, Wu T, Miao M, Wang J, Weng X, Ferber JR, Herrinton LJ, Zhu Q, Gao E, Checkoway H, Yuan W. Occupational exposure to bisphenol-A (BPA) and the risk of self-reported male sexual dysfunction. *Human Reproduction*. 2009;**25**:19–27. DOI: 10.1093/humrep/dep381
- [13] Kundakovic M, Champagne FA. Epigenetic perspective on the developmental effects of bisphenol A. *Brain, Behavior, and Immunity*. 2011;**25**:1084–1093. DOI: 10.1016/j.bbi.2011.02.005
- [14] Singh S, Li SS. Epigenetic effects of environmental chemicals bisphenol A and phthalates. *International Journal of Molecular Sciences*. 2012;**13**:10143–10153. DOI: 10.3390/ijms130810143
- [15] Shin BS, Kim CH, Jun YS, Kim DH, Lee BM, Yoon CH, Park EH, Lee KC, Han SY, Park KL, Kim HS, Yoo SD. Physiologically based pharmacokinetics of BPA. *Journal of Toxicology and Environmental Health A*. 2004;**67**:1971–1985. DOI: 10.1080/15287390490514615
- [16] Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, Soto AM. Bisphenol-A and the great divide: A review of controversies in the field of endocrine disruption. *Endocrine Reviews*. 2009;**30**:75–95. DOI: 10.1210/er.2008-0021

- [17] Huleihel M, Lunenfeld E. Regulation of spermatogenesis by paracrine/autocrine testicular factors. *Asian Journal of Andrology*. 2004;**6**:259–268
- [18] Diemer T, Allen JA, Hales KH, Hales DB. Reactive oxygen disrupts mitochondria in MA-10 tumor Leydig cells and inhibits steroidogenic acute regulatory (StAR) protein and steroidogenesis. *Endocrinology*. 2003;**144**:2882–2891. DOI: 10.1210/en.2002-0090
- [19] Labohá P, Jambor T, Yawer A, Lukáč N, Sovadinová I. Molecular mechanisms of alkylphenol-mediated endocrine disruption in Leydig cells. *Toxicology Letters*. 2016;**258**:245–246. DOI: 10.1016/j.toxlet.2016.06.1872
- [20] Tsai M, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annual Review of Biochemistry*. 1994;**63**:451–486. DOI: 10.1146/annurev.biochem.63.1.451
- [21] Danzo BJ. Environmental xenobiotics may disrupt normal endocrine function by interfering with the binding of physiological ligand to steroid receptors and binding proteins. *Environmental Health Perspectives*. 1997;**105**:294–301. DOI: 10.2307/3433266
- [22] Bolger R, Wiese TE, Ervin K, Nestich S, Checovich W. Rapid screening of environmental chemicals for estrogen receptor binding capacity. *Environmental Health Perspectives*. 1998;**106**:551–557. DOI: 10.2307/3434229
- [23] Gould JC, Leonar LS, Maness SC, Wagner BL, Conner K, Zacharewski T, Safe S, McDonnell DP, Gaido KW. Bisphenol A interacts with the estrogen receptor alpha in a distinct manner from estradiol. *Molecular and Cellular Endocrinology*. 1998;**142**:203–214. DOI: 10.1016/s0303-7207(98)00084-7
- [24] Tora L, White J, Brou Ch, Tasset D, Webster N, Scheer E, Chambon P. The human estrogen receptor has two independent nonacidic transcriptional activation functions. *Cell*. 1989;**59**:477–487. DOI: 10.1016/0092-8674(89)90031-7
- [25] Pennie WD, Aldridge TC, Brooks AN. Differential activation by xenoestrogens of ER α and ER β when linked to different response elements. *Journal of Endocrinology*. 1998;**158**:11–14. DOI: 10.1677/joe.0.158r011
- [26] Routledge EJ, White R, Parker MG, Sumpter JP. Differential effects of xenoestrogens on coactivator recruitment by estrogen receptor (ER) alpha and ER beta. *The Journal of Biological Chemistry*. 2000;**275**:35986–35993. DOI: 10.1074/jbc.m006777200
- [27] Song KH, Lee K, Choi HS. Endocrine disrupter bisphenol A induces orphan nuclear receptor Nur77 gene expression and steroidogenesis in mouse testicular Leydig cells. *Endocrinology*. 2002;**143**:2208–2215. DOI: 10.1210/endo.143.6.8847
- [28] An BS, Kang SK, Shin JH, Jeung EB. Stimulation of calbindin-D(9k) mRNA expression in the rat uterus by octylphenol, nonylphenol and bisphenol. *Molecular and Cellular Endocrinology*. 2002;**191**:177–186. DOI: 10.1016/s0303-7207(02)00042-4
- [29] Vivacqua A, Recchia AG, Fasanella G, Gabriele S, Carpino A, Rago V, Di Gioia ML, Leggio A, Bonofiglio D, Liguori A, Maggiolini M. The food contaminants bisphenol A

and 4-nonylphenol act as agonists for estrogen receptor alpha in MCF7 breast cancer cells. *Endocrine*. 2003;**22**:275–284. DOI: 10.1385/endo:22:3:275

- [30] Rankouhi R, Sanderson JT, Van Holsteijn I, Van Kooten P, Bosveld AT, Van den Berg M. Effects of environmental and natural estrogens on vitellogenin production in hepatocytes of the brown frog (*Rana temporaria*). *Aquatic Toxicology*. 2005;**71**:97–101. DOI: 10.1016/j.aquatox.2004.09.009
- [31] Wersinger SR, Haisenleder DJ, Lubahn DB, Rissman EF. Steroid feedback on gonadotropin release and pituitary gonadotropin subunit mRNA in mice lacking a functional estrogen receptor alpha. *Endocrine*. 1999;**11**:137–143. DOI: 10.1385/endo:11:2:137
- [32] Sohoni P, Sumpter JP. Several environmental oestrogens are also anti-androgens. *Journal of Endocrinology*. 1998;**158**:327–339. DOI: 10.1677/joe.0.1580327
- [33] Gaido KW, Maness SC, McDnnell DP, Dehal SS, Kupfer D, Safe S. Interaction of methoxychlor and related compounds with estrogen receptor alpha and beta, and androgen receptor: Structure-activity studies. *Molecular Pharmacology*. 2000;**58**:852–858. DOI: 10.1124/mol.58.4.852
- [34] Wong CH, Zhou ZX, Sar M, Wilson EM. Steroid requirement for androgen receptor dimerization and DNA binding. *The Journal of Biological Chemistry*. 1993;**268**:19004–19012
- [35] McKenna NJ, Lanz RB, O'Malley BW. Nuclear receptor coregulators: Cellular and molecular biology. *Endocrine Reviews*. 1999;**20**:321–344. DOI: 10.1210/er.20.3.321
- [36] Yeh S, Chang C. Cloning and characterization of a specific coactivator, ARA70, for the androgen receptor in human prostate cells. *Proceedings of the National Academy of Sciences*. 1996;**93**:5517–5521. DOI: 10.1073/pnas.93.11.5517
- [37] Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS. Androgen receptor defects: Historical, clinical, and molecular perspectives. *Endocrine Reviews*. 1995;**16**:271–321. DOI: 10.1210/er.16.3.271
- [38] Lee HJ, Chattopadhyay S, Gong EY, Ahn RS, Lee K. Antiandrogenic effects of bisphenol A and nonylphenol on the function of androgen receptor. *Toxicological Sciences*. 2003;**75**:40–46. DOI: 10.1093/toxsci/kfg150
- [39] Sun H, Xu LC, Chen JF, Song L, Wang XR. Effect of bisphenol A, tetrachlorobisphenol A and pentachlorophenol on the transcriptional activities of androgen receptor-mediated reporter gene. *Food and Chemical Toxicology*. 2006;**44**:1916–1921. DOI: 10.1016/j.fct.2006.06.013
- [40] Kitamura S, Suzuki T, Sanoh S, Kohta R, Jinno N, Suigihara K, Yoshihara S, Fujimota N, Watanabe H, Ohta S. Comparative study of the endocrine-disrupting activity of bisphenol A and 19 related compounds. *Toxicological Sciences*. 2005;**84**:249–259. DOI: 10.1093/toxsci/kfi074
- [41] Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinänen R, Palmberg Ch, Palotie A, Tammela T, Isola J, Kallioniemi OP. *In vivo* amplification of the androgen receptor

- gene and progression of human prostate cancer. *Nature Genetics*. 1995;**9**:401–406. DOI: 10.1038/ng0495-401
- [42] Ramos JG, Varayoud J, Sonnenschein C, Soto AM, Toro MM, Luque EH. Prenatal exposure to low doses of bisphenol A alters the periductal stroma and glandular cell function in the rat ventral prostate. *Biology of Reproduction*. 2001;**65**:1271–1277. DOI: 10.1095/biolreprod65.4.1271
- [43] Cagen SZ, Waechter JM, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, Harris LR. Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A. *Toxicological Sciences*. 1999;**50**:36–44. DOI: 10.1093/toxsci/50.1.36
- [44] Takahashi O, Oishi S. Disposition of orally administered 2,2-bis (4-hydroxyphenyl) propane (BPA) in pregnant rats and the placental transfer to fetuses. *Environmental Health Perspectives*. 2000;**108**:931–935. DOI: 10.2307/3435050
- [45] Hughes PJ, McLellan H, Lowes DA, Kahn SZ, Bilmen JG, Tovey SC, Godfrey RE, Michell RH, Kirk CJ, Michelangeli F. Estrogenic alkylphenols induce cell death by inhibiting testis endoplasmic reticulum $CA(2^+)$ pumps. *Biochemical and Biophysical Research Communications*. 2000;**277**:568–574. DOI: 10.1006/bbrc.2000.3710
- [46] Sanderson JT, Seinen W, Giesy JP, van den Berg M. 2-chloro-s-triazine herbicides induce aromatase (CYP19) activity in H295R human adrenocortical carcinoma cells: A novel mechanism for estrogenicity. *Toxicological Sciences*. 2000;**54**:121–127. DOI: 10.1093/toxsci/54.1.121
- [47] Hilscherova K, Jones PD, Gracia T, Newsted JL, Zhang XW, Sanderson JT, Yu RMK, Wu RSS, Giesy JP. Assessment of the effects of chemicals on the expression of ten steroidogenic genes in the H295R cell line using real-time PCR. *Toxicological Sciences*. 2004;**81**:78–89. DOI: 10.1093/toxsci/kfh191
- [48] Zhang X, Yu RM, Jones PD, Lam GK, Newsted JL, Gracia T, Hecker M, Hilscherova K, Sanderson T, Wu RS, Giesy JP. Quantitative RT-PCR methods for evaluating toxicant-induced effects on steroidogenesis using the H295R cell line. *Environmental Science and Technology*. 2005;**39**:2777–2785. DOI: 10.1021/es048679k
- [49] Zhang X, Chang H, Wiseman S, He Y, Higley E, Jones P, Wong CK, Al-Khedhairi A, Giesy JP, Hecker M. Bisphenol A disrupts steroidogenesis in human H295R cells. *Toxicological Sciences*. 2011;**121**:320–327. DOI: 10.1093/toxsci/kfr061
- [50] Akingbemi BT, Sottas CHM, Koulova AI, Klinefelter GR, Hardy MP. Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. *Endocrinology*. 2004;**145**:592–603. DOI: 10.1210/en.2003-1174
- [51] Ye X, Wong LY, Bishop AM, Calafat AM. Variability of urinary concentrations of bisphenol A in spot samples, first-morning voids, and 24-hour collections. *Environmental Health Perspectives*. 2011;**119**:983–988. DOI: 10.1289/ehp.1002701

- [52] Geissler WM, Davis DL, Wu L, Bradshaw KD, Patel S, Mendonca BB, Elliston KO, Wilson JD, Russell DW, Andersson S. Male pseudohermaphroditism caused by mutations of testicular 17 beta-hydroxysteroid dehydrogenase 3. *Nature Genetics*. 1994;**7**:34–39. DOI: 10.1038/ng0594-34
- [53] Andersson S, Moghrabi N. Physiology and molecular genetics of 17 beta-hydroxysteroid dehydrogenases. *Steroids*. 1997;**62**:143–147. DOI: 10.1016/s0039-128x(96)00173-0
- [54] Hess RA. Oestrogen in fluid transport in efferent ducts of the male reproductive tract. *Reviews of Reproduction*. 2000;**5**:84–92. DOI: 10.1530/revreprod/5.2.84
- [55] Kinoshita Y, Chen S. Induction of aromatase (CYO19) expression in breast cancer cells through a nongenomic action of estrogen receptor α . *Cancer Research*. 2003;**63**:3546–3555
- [56] Nikula H, Talonpoika T, Kaleva M, Toppari J. Inhibition of hCG-stimulated steroidogenesis in cultured mouse Leydig tumor cells by BPA and octylphenols. *Toxicology and Applied Pharmacology*. 1999;**157**:166–173. DOI: 10.1006/taap.1999.8674
- [57] Gaskell TL, Robinson LL, Groome, NP, Anderson RA, Saunders PT. Differential expression of two estrogen receptor- β isoforms in the human fetal testis during the second trimester of pregnancy. *The Journal of Clinical Endocrinology and Metabolism*. 2003;**88**:424–432. DOI: 10.1210/jc.2002-020811
- [58] Schönfelder G, Wittfoht W, Hopp H, Talsness CHE, Paul M, Chahoud I. Parental bisphenol A accumulation in the human maternal-fetal-placental unit. *Environmental Health Perspectives*. 2002;**110**:703–707. DOI: 10.1289/ehp.021100703
- [59] Ikezuki Y, Tsutsumi O, Takai Y, Kamei Y, Taketani Y. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. *Human Reproduction*. 2002;**17**:2839–2841. DOI: 10.1093/humrep/17.11.2839
- [60] Uchida K, Suzuki A, Kobayashi Y, Buchanan DL, Sato T, Watanabe H, Katsu Y, Suzuki J, Asaoka K, Mori Ch, Arizono K, Iguchi T. Bisphenol A administration during pregnancy results in fetal exposure in mice and monkeys. *Journal of Health Science*. 2002;**48**:579–582. DOI: 10.1248/jhs.48.579
- [61] Kawai K, Nozaki T, Nishikata H, Aou S, Takii M, Kubo Ch. Aggressive behavior and serum testosterone concentration during the maturation process of male mice: The effects of fetal exposure to bisphenol A. *Environmental Health Perspectives*. 2003;**111**:175–178. DOI: 10.1289/ehp.5440
- [62] Nagel SC, Vom Saal FS, Thayer KA, Dhar MG, Boechler M, Welshons WV. Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative *in vivo* bioactivity of the xenoestrogens bisphenol A and octylphenol. *Environmental Health Perspectives*. 1997;**105**:70–76. DOI: 10.2307/3433065
- [63] Vom Saal FS, Cooke PS, Buchanan DL, Palanza P, Thayer KA, Nagel SC, Parmigiani S, Welshons WV. A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. *Toxicology and Industrial Health*. 1998;**14**:239–260. DOI: 10.1177/074823379801400115

- [64] Xi W, Wan HT, Zhao YG, Wong MH, Giesy JP, Wong CK. Effects of perinatal exposure to bisphenol A and di(2-ethylhexyl)-phthalate on gonadal development of male mice. *Environmental Science and Pollution Research International*. 2012;**19**:2515–2527. DOI: 10.1007/s11356-012-0827-y
- [65] Takeuchi T, Tsutsumi O. Serum bisphenol A concentrations showed gender differences, possibly linked to androgen levels. *Biochemical and Biophysical Research Communications*. 2002;**291**:76–78. DOI: 10.1006/bbrc.2002.6407
- [66] Tanaka M, Nakaya S, Katayama M, Leffers H, Nozawa S, Nakazawa R, Iwamoto T, Kobayashi S. Effect of prenatal exposure to bisphenol A on the serum testosterone concentration of rats at birth. *Human and Experimental Toxicology*. 2006;**25**:369–373. DOI: 10.1191/0960327106ht638oa
- [67] Nanjappa MK, Simon L, Akingbemi BT. The industrial chemical bisphenol A (BPA) interferes with proliferative activity and development of steroidogenic capacity in rat Leydig cells. *Biology of Reproduction*. 2012;**86**:1–12. DOI: 10.1095/biolreprod.111.095349
- [68] Timms BG, Petersen SL, Vom Saal FS. Prostate gland growth during development is stimulated in both male and female rat fetuses by intrauterine proximity to female fetuses. *The Journal of Urology*. 1999;**161**:1694–1701. DOI: 10.1016/s0022-5347(05)69007-6
- [69] Marker PC, Donjacour AA, Dahiya R, Cunha GR. Hormonal, cellular, and molecular control of prostatic development. *Developmental Biology*. 2003;**253**:165–174. DOI: 10.1016/s0012-1606(02)00031-3
- [70] Adam BL, Qu Y, Davis JW, Ward MD, Clements MA, Cazares LH, Semmes OJ, Schellhammer PF, Yasui Y, Feng Z, Wright GL. Serum protein fingerprinting coupled with a pattern-matching algorithm distinguishes prostate cancer from benign prostate hyperplasia and healthy men. *Cancer Research*. 2002;**62**:3609–3614
- [71] Alonso-Magdalena P, Vieira E, Soriano S, Menes L, Burks D, Quesada I, Nadal A. Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mother and adult male offspring. *Environmental Health Perspectives*. 2010;**118**:1243–1250. DOI: 10.1289/ehp.1001993
- [72] Kloas W, Lutz I, Einspanier R. Amphibians as a model to study endocrine disruptors: II. Estrogenic activity of environmental chemicals *in vitro* and *in vivo*. *The Science of the Total Environment*. 1999;**225**:59–68. DOI: 10.1016/s0048-9697(98)00332-5
- [73] Miyata S, Koike S, Kubo T. Hormonal reversal and the genetic control of sex differentiation in *Xenopus*. *Zoological Science*. 1999;**16**:335–340. DOI: 10.2108/zsj.16.335
- [74] Bögi C, Levy G, Lutz I, Kloas W. Functional genomics and sexual differentiation in amphibians. *Comparative biochemistry and Physiology. Part B , Biochemistry & Molecular Biology*. 2002;**133**:559–570. DOI: 10.1016/s1096-4959(02)00162-8
- [75] Yokota H, Tsuruda Y, Maeda M, Oshima Y, Tadokoro H, Nakazono A, Honjo T, Kobayashi K. Effect of bisphenol A on the early life stage in Japanese medaka (*Oryzias latipes*). *Environmental Toxicology and Chemistry*. 2000;**19**:1925–1930. DOI: 10.1002/etc.5620190730

- [76] Ashfield LA, Pottinger TG, Sumpter JP. Exposure of female juvenile rainbow trout to alkylphenolic compounds results in modifications to growth and ovosomatic index. *Environmental Toxicology*. 1998;**17**:679–686. DOI: 10.1897/1551-5028(1998)017<0679:eofjrt>2.3.co;2
- [77] Pryor JL, Hughes C, Foster W, Hales BF, Rodaire B. Critical windows of exposure for children's health: The reproductive system in animals and humans. *Environmental Health Perspectives*. 2000;**108**:491–503. DOI: 10.2307/3454541
- [78] Aoki T, Takada T. Bisphenol A modulates germ cell differentiation and retinoic acid signaling in mouse ES cells. *Reproductive Toxicology*. 2012;**34**:463–470. DOI: 10.1016/j.reprotox.2012.06.001
- [79] Morrissey RE, Lamb JC, Schwetz BA, Teague JL, Morris RW. Association of sperm, vaginal cytology, and reproductive organ weight data with results of continuous breeding reproduction studies in Swiss (CD-1) mice. *Fundamental and Applied Toxicology*. 1988;**11**:359–371. DOI: 10.1016/0272-0590(88)90160-1
- [80] Manfo FPT, Jubendradass R, Nantia EA, Moundipa PF, Mathur PP. Adverse effects of bisphenol A on male reproductive function. *Reviews of Environmental Contamination and Toxicology*. 2014;**228**:57–82. DOI: 10.1007/978-3-319-01619-1_3
- [81] Kato H, Furuhashi T, Tanaka M, Katsu Y, Watanabe H, Ohta Y, Iguchi T. Effects of BPA given neonatally on reproductive functions of male rats. *Reproductive Toxicology*. 2006;**22**:20–29. DOI: 10.1016/j.reprotox.2005.10.003
- [82] Wozniak AL, Bulayeva NN, Watson CS. Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor- α -mediated Ca^{2+} fluxes and prolactin release in GH3/B6 pituitary tumor cells. *Environmental Health Perspectives*. 2005;**113**:431–439. DOI: 10.1289/ehp.7505
- [83] Sakaue M, Ohsako S, Ishimura R, Kurosawa S, Kurohmaru M, Hayashi Y, Aoki Y, Yonemoto J, Tohyama C. Bisphenol-A affects spermatogenesis in the adult rat even at low dose. *Journal of Occupational Health*. 2001;**43**:185–190. DOI: 10.1539/joh.43.185
- [84] Míñquez-Alarcón L, Hauser R, Gaskins AJ. Effects of bisphenol A on male and couple reproductive health: A review. *Fertility and Sterility*. 2016;**106**:864–870. DOI: 10.1016/j.fertnstert.2016.07.1118
- [85] Mathur PP, D'Cruz SC. The effect of environmental contaminants on testicular function. *Asian Journal of Andrology*. 2011;**13**:585–591. DOI: 10.1038/aja.2011.40
- [86] Kabuto H, Amakawa M, Shishibori T. Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. *Life Sciences*. 2004;**74**:2931–2940. DOI: 10.1016/j.lfs.2003.07.060
- [87] Hulak M, Gazo I, Shaliutina A, Linhartova P. *In vitro* effects of BPA on the quality parameters, oxidative stress, DNA integrity and adenosine triphosphate content in sterlet (*Acipenser ruthenus*) spermatozoa. *Comparative Biochemistry and Physiology*. 2013;**158**:64–71. DOI: 10.1016/j.cbpc.2013.05.002

- [88] D'Cruz SC, Jubendradass R, Mathur PP. Bisphenol A induces oxidative stress and decreases levels of insulin receptor substrate 2 and glucose transporter 8 in rat testis. *Reproductive Sciences*. 2012;**19**:163–172. DOI: 10.1177/1933719111415547
- [89] Rubin BS, Murray MK, Damassa DA, King JC, Soto AM. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. *Environmental Health Perspectives*. 2001;**109**:675–680. DOI: 10.2307/3454783
- [90] Jin P, Wang X, Chang F, Bai Y, Li Y, Zhou R, Chen L. Low dose bisphenol A impairs spermatogenesis by suppressing reproductive hormone production and promoting germ cell apoptosis in adult rats. *Journal of Biomedical Research*. 2013;**27**:135–144. DOI: 10.7555/jbr.27.20120076
- [91] Gurmeet KSS, Rosnah I, Normadiah MK, Das S, Mustafa AM. Detrimental effects of BPA on development and functions of the male reproductive system in experimental rats. *Experimental and Clinical Sciences Journal* 2014;**13**:151–160
- [92] Furuya M, Adachi K, Kuwahara S, Ogawa K, Tsukamoto Y. Inhibition of male chick phenotypes and spermatogenesis by bisphenol-A. *Life Sciences*. 2006;**78**:1767–1776. DOI: 10.1016/j.lfs.2005.08.016
- [93] Li D, Zhou Z, Miao M, He Y, Wang J, Ferber J, Herrinton LJ, Gao E, Yuan W. Urine bisphenol-A (BPA) level in relation to semen quality. *Fertility and Sterility*. 2011;**95**:625–630. DOI: 10.1016/j.fertnstert.2010.09.026
- [94] Wang T, Lu J, Xu M, Xu Y, Li M, Liu Y, Tian X, Chen Y, Dai M, Wang W, Lai S, Bi Y, Ning G. Urinary bisphenol A concentration and thyroid function in Chinese adults. *Epidemiology*. 2013;**24**:295–302. DOI: 10.1097/ede.0b013e318280e02f
- [95] Bennetts LE, De Iuliis GN, Nixon B, Kime M, Zelski K, McVicar CM, Lewis SE, Aitken RJ. Impact of estrogenic compounds on DNA integrity in human spermatozoa: Evidence for cross-linking and redox cycling activities. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 2008;**641**:1–11. DOI: 10.1016/j.mrfmmm.2008.02.002
- [96] Martinez C, Mar C, Azcarate M, Pascual P, Aritzeta JM, Lopez-Urrutia A. Sperm motility index: A quick screening parameter from sperm quality analyser-IIB to rule out oligo- and asthenozoospermia in male fertility study. *Human Reproduction*. 2000;**15**:1727–1733. DOI: 10.1093/humrep/15.8.1727
- [97] Jansen RPS, Burton GJ. Mitochondrial dysfunction in reproduction. *Mitochondrion*. 2004;**4**:577–600. DOI: 10.1016/j.mito.2004.07.038
- [98] Kotwicka M, Skibinska I, Jendraszak M, Jedrzejczak P. 17 β -estradiol modifies human spermatozoa mitochondrial function in vitro. *Reproductive Biology and Endocrinology*. 2016;**14**:50. DOI: 10.1186/s12958-016-0186-5
- [99] Anjum S, Rahman A, Kaur M, Ahmad F, Rashid H, Ansari RA, Raisuddin S. Melatonin Ameliorates BPA-induced biochemical toxicity in testicular mitochondria of mouse. *Food and Chemical Toxicology*. 2011;**49**:2849–2854. DOI: 10.1016/j.fct.2011.07.062

- [100] Al-Hiyasat AS, Darmani H, Elbetieha AM. Effects of bisphenol A on adult male mouse fertility. *European Journal of Oral Sciences*. 2002;**110**:163–167. DOI: 10.1034/j.1600-0722.2002.11201.x
- [101] Dobrzyńska MM, Radzikowska J. Genotoxicity and reproductive toxicity of bisphenol A and X-ray/bisphenol A combination in male mice. *Drug and Chemical Toxicology*. 2013;**36**:19–26. DOI: 10.3109/01480545.2011.644561
- [102] Singh RP, Shafeeque CM, Sharma SK, Pandey NK, Singh R, Mohan J, Kolluri G, Saxena M, Sharma B, Sastry KV, Kataria JM, Azeez PA. Bisphenol A reduces fertilizing ability and motility by compromising mitochondrial function of sperm. *Environmental Toxicology and Chemistry*. 2015;**34**:1617–1622. DOI: 10.1002/etc.2957
- [103] Deutschmann A, Hans M, Meyer R, Häberlein H, Swandulla D. Bisphenol A inhibits voltage-activated Ca²⁺ channels *in vitro*: Mechanisms and structural requirements. *Molecular Pharmacology*. 2013;**83**:501–511. DOI: 10.1124/mol.112.081372
- [104] Lukacova J, Jambor T, Knazicka Z, Tvrda E, Kolesarova A, Lukac N. Dose- and time-dependent effects of BPA on bovine spermatozoa *in vitro*. *Journal of Environmental Science and Health*. 2015;**50**:669–676. DOI: 10.1080/10934529.2015.1011963
- [105] Rahman MS, Kwon WS, Lee JS, Yoon SJ, Ryu BY, Pang MG. Bisphenol-A affects male fertility via fertility-related proteins in spermatozoa. *Scientific Reports*. 2015;**5**:9169. DOI: 10.1038/srep09169
- [106] Hatef A, Alavi SMH, Linhartova Z, Rodina M, Policar T, Linhart O. *In vitro* effects of BPA on sperm motility characteristics perca fluviatilis L. (Percidae; Teleostei). *Journal of Applied Ichthyology*. 2010;**26**:696–701. DOI: 10.1111/j.1439-0426.2010.01543.x
- [107] Meeker JD, Ehrlich S, Toth TL, Wright DL, Calafat AM, Trisini AT, Ye X, Hauser R. Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. *Reproductive Toxicology*. 2010;**30**:532–539. DOI: 10.1016/j.reprotox.2010.07.005
- [108] Lassen TH, Frederiksen H, Jensen TK, Petersen JH, Joensen UN, Main KM, Skakkebaek NE, Juul A, Jorgensen N, Andersson A. Urinary bisphenol A levels in young men: Association with reproductive hormones and semen quality. *Environmental Health Perspectives*. 2014;**122**:478–484. DOI: 10.1289/ehp.1307309
- [109] Iwakura T, Iwafuchi M, Muraoka D, Yokosuka M, Shiga T, Watanabe C, Ohtani-Kaneko R. *In vitro* effects of bisphenol A on developing hypothalamic neurons. *Toxicology*. 2010;**272**:52–58. DOI: 10.1016/j.tox.2010.04.005
- [110] Miao M, Yuan W, He Y, Zhou Z, Wang J, Gao E, Li G, Li D. In utero exposure to bisphenol-A and anogenital distance of male offspring. *Birth Defects Research*. 2011;**91**:867–872. DOI: 10.1002/bdra.22845
- [111] Gupta CH. Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. *Proceedings of the Society for Experimental Biology and Medicine*. 2000;**224**:61–68. DOI: 10.1046/j.1525-1373.2000.22402.x

- [112] Chahoud I, Fialkowski O, Gericke CH, Merker H, Talsness CE. The effects of low and high doses of bisphenol A on the reproductive system of female and male rat offspring. *Reproductive Toxicology*. 2000;**40**:587–599. DOI: 10.1016/S0890-6238(01)00153-8
- [113] Salian S, Doshi T, Vanage G. Perinatal exposure of rats to bisphenol A affects fertility of male offspring-an overview. *Reproductive Toxicology*. 2011;**31**:359–362. DOI: 10.1016/j.reprotox.2010.10.008
- [114] Griswold MD. Interactions between germ cells and Sertoli cells in the testis. *Biology of Reproduction*. 1995;**52**:211–216. DOI: 10.1095/biolreprod52.2.211
- [115] Iida H, Maehara K, Doiguchi M, Mori T, Yamada F. Bisphenol A-induced apoptosis of cultured rat Sertoli cells. *Reproductive Toxicology*. 2003;**17**:457–464. DOI: 10.1016/s0890-6238(03)00034-0
- [116] Tabuchi Y, Konodo T. cDNA microarray analysis reveals chop-10 plays a key role in Sertoli cell injury induced by bisphenol A. *Biochemical and Biophysical Research Communications*. 2003;**305**:54–61. DOI: 10.1016/s0006-291x(03)00708-3
- [117] Toyama Y, Suzuki-Toyota F, Maekawa M, Ito C, Toshimori K. Adverse effects of bisphenol A to spermatogenesis in mice and rats. *Archives of Histology and Cytology*. 2004;**67**:373–381. DOI: 10.1679/aohc.67.373
- [118] Fiorini C, Tilloy-Ellul A, Chevalier S, Charusel C, Pointis G. Sertoli cell junctional proteins as early targets for different classes of reproductive toxicants. *Reproductive Toxicology*. 2004;**18**:413–421. DOI: 10.1016/j.reprotox.2004.01.002
- [119] Tainaka H, Takahashi H, Umezawa M, Tanaka H, Nishimune Y, Oshio S, Takeda K. Evaluation of the testicular toxicity of prenatal exposure to bisphenol A based on microarray analysis combined with MeSH annotation. *The Journal of Toxicological Sciences*. 2012;**37**:539–548. DOI: 10.2131/jts.37.539
- [120] Su L, Mruk DD, Cheng CY. Drug transporters, the blood-testis barrier, and spermatogenesis. *Journal of Endocrinology*. 2011;**208**:207–223. DOI: 10.5353/th_b4775281
- [121] Griveau JF, Dumont E, Renard P, Callegari JP, Le Lannou D. Reactive oxygen species, lipid peroxidation and enzymatic defence systems in human spermatozoa. *The Journal of the Society for Reproduction and Fertility*. 1995;**103**:17–26. DOI: 10.1530/jrf.0.1030017
- [122] Aitken RJ, Clarkson JS, Fishel S. Generation of reactive oxygen species, lipid peroxidation, and human sperm function. *Biology of Reproduction*. 1989;**41**:183–197. DOI: 10.1095/biolreprod41.1.183
- [123] El-Beshbishy HA, Aly HAA, El-Shafey M. Lipoic acid mitigates bisphenol A-induced testicular mitochondrial toxicity in rats. *Toxicology and Industrial Health*. 2013;**29**:875–887. DOI: 10.1177/0748233712446728
- [124] De Flora S, Micale RT, La Maestra S, Izzotti A, D'Agostini F, Camoirano A, Davoli SA, Troglio MG, Rizzi F, Davalli P, Bettuzzi S. Upregulation of clusterin in prostate and DNA damage in spermatozoa from bisphenol A-treated rats and formation of DNA

- adducts in cultured human prostatic cells. *Toxicological Sciences*. 2011;**122**:45–51. DOI: 10.1093/toxsci/kfr096
- [125] Barbonetti A, Castellini C, Di Giammarco N, Santilli G, Francavilla S, Francavilla F. *In vitro* exposure of human spermatozoa to BPA induces pro-oxidative/apoptotic mitochondrial dysfunction. *Reproductive Toxicology*. 2016;**66**:61–67. DOI: 10.1016/j.reprotox.2016.09.014
- [126] Atkinson A, Roy D. *In vitro* conversion of environmental estrogenic chemical bisphenol A to DNA binding metabolite(s). *Biochemical and Biophysical Research Communications*. 1995;**210**:424–433. DOI: 10.1006/bbrc.1995.1678
- [127] Jonathan N, Steinmets R. Xenoestrogens: The emerging story of bisphenol A. *Trends in Endocrinology and Metabolism*. 1998;**9**:124–128. DOI: 10.1016/s1043-2760(98)00029-0
- [128] Atkinson A, Roy D. *In vivo* DNA adduct formation by bisphenol A. *Environmental and Molecular Mutagenesis*. 1995;**26**:60–66. DOI: 10.1002/em.2850260109
- [129] Knaak JB, Sullivan LJ. Metabolism of bisphenol A in the rat. *Toxicology and Applied Pharmacology*. 1966;**8**:175–184. DOI: 10.1016/s0041-008x(66)80001-7
- [130] Tiwari D, Vanage G. Mutagenic effect of bisphenol A on adult rat male germ cells and their fertility. *Reproductive Toxicology*. 2013;**40**:60–68. DOI: 10.1016/j.reprotox.2013.05.013
- [131] Mileva G, Baker SL, Konkole ATM, Bielajew C. Bisphenol-A: Epigenetic reprogramming and effects on reproduction and behavior. *International Journal of Environmental Research and Public Health*. 2014;**11**:7537–7561. DOI: 10.3390/ijerph110707537
- [132] Farabollini F, Porrini S, Dessi-Fulgheri F. Perinatal exposure to the estrogenic pollutant bisphenol A affects behavior in male and female rats. *Pharmacology Biochemistry and Behavior*. 1999;**64**:687–694. DOI: 10.1016/s0091-3057(99)00136-7
- [133] Jones BA, Watson NV. Perinatal BPA exposure demasculinizes males in measures of affect but has no effect on water maze learning in adulthood. *Hormones and Behavior*. 2012;**61**:605–610. DOI: 10.1016/j.yhbeh.2012.02.011
- [134] Della Seta D, Minder I, Belloni V, Aloisi AM, Dessi-Fulgheri F, Farabollini F. Pubertal exposure to estrogenic chemicals affects behavior in juvenile and adult male rats. *Hormones and Behavior*. 2006;**50**:301–307. DOI: 10.1016/j.yhbeh.2006.03.015
- [135] Manikkam M, Guerrero-Bosagna C, Tracey R, Haque M, Skinner MK. Transgenerational actions of environmental compounds on reproductive disease and identification of epigenetic biomarkers of ancestral exposures. *PLoS One*. 2012;**7**:31901. DOI: 10.1371/journal.pone.0031901
- [136] Skinner MK, Guerrero-Bosagna C, Haque M, Nilsson E, Bhandari R, McCarrey JR. Environmentally induced transgenerational epigenetic reprogramming of primordial germ cells and the subsequent germ line. *PLoS One*. 2013;**8**:66318. DOI: 10.1371/journal.pone.0066318

